E. Preparation and Characterization of Gold Nanoparticles

Gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ as described in Example 1. Transmission Electron Microscopy (TEM) performed with a Hitachi 8100 TEM was used to determine the size distribution of the resulting nanoparticles. At least 250 particles were sized from TEM negatives using graphics software (ImageTool). The average diameter of a typical particle preparation was 15.7 ± 1.2 nm. Assuming spherical nanoparticles and density equivalent to that of bulk gold (19.30 g/cm^2), an average molecular weight per particle was calculated (2.4 x 10^7 g/mol). The atomic gold concentration in a solution of gold nanoparticles was determined by ICP-AES (inductively coupled plasmon atomic emission spectroscopy). A gold atomic absorption standard solution (Aldrich) was used for calibration. Comparison of atomic gold concentration in the particle solution to the average particle volume obtained by TEM analysis yielded the molar concentration of gold particles in a given preparation, typically ~10 nM. By measuring the UV-vis absorbance of nanoparticle solutions at the surface plasmon frequency (520 nm), the molar extinction coefficients (ε at 520 nm) were calculated for the particles, typically 4.2 x 10^8 M^{-1} cm^{-1} for 15.7 ± 1.2 nm diameter particles.

F. Preparation of Gold Thin Films.

Silicon wafers were cut into ~10 mm x 6 mm pieces and cleaned with piranha etch solution (4:1 concentrated H_2SO_4: 30% H_2O_2) for 30 min at 50 °C, then rinsed with copious amounts of water, followed by ethanol. (*Warning: piranha etch solution reacts violently with organic materials and should be handled with extreme caution.)* Metal was deposited at a rate of 0.2 nm/s using an Edwards Auto 306 evaporator (base pressure of 3 x 10^{-7} millibar) equipped with an Edwards FTM6 quartz crystal microbalance. The oxidized sides of the silicon were coated with a Ti adhesion layer of 5 nm, followed by 200 nm of gold..

G. Preparation of 5' Alkylthiol Oligonucleotide-Modified Gold Nanoparticles.

Gold nanoparticles were modified with fluorescein-alkylthiol oligonucleotides by adding freshly deprotected oligonucleotides to aqueous nanoparticle solution (particle concentration ~10 nM) to a final oligonucleotide concentration of 3 μM. After 24 hours, the

solution was buffered at pH 7 (0.01 M phosphate), and NaCl solution was added (to final concentration of 0.1 M). The solution was allowed to 'age' under these conditions for an additional 40 hours. Excess reagents were then removed by centrifugation for 30 minutes at 14,000 rpm. Following removal of the supernatant, the red oily precipitate was washed twice with 0.3 M NaCl, 10 mM phosphate buffer, pH 7, solution (PBS) by successive centrifugation and redispersion, then finally redispersed in fresh buffer solution. Invariably, a small amount (~ 10% as determined by UV-vis spectroscopy) of nanoparticle is discarded with the supernatant during the washing procedure. Therefore, final nanoparticle concentrations were determined by TEM, ICP-AES, and UV-vis spectroscopy (see above). Extinction coefficients and particle size distributions did not change significantly as a result of the oligonucleotide modification.

H. Preparation of 5' Alkylthiol Oligonucleotide-Modified Gold Thin Films.

Silicon supported gold thin films were immersed in deposition solutions of deprotected alkylthiol modified oligonucleotides for equal times and buffer conditions as for the gold nanoparticles. Following oligonucleotide deposition, the films were rinsed extensively with 0.3 M PBS and stored in buffer solution. Gold was evaporated on one side only, leaving an unpassivated silicon/silicon oxide face. However, alkylthiol modified DNA did not adsorb appreciably to bare silicon oxide surfaces that were rinsed with PBS.

I. Quantitation of Alkylthiol-Oligonucleotides Loaded on Nanoparticles.

Mercaptoethanol (ME) was added (final concentration 12 mM) to fluorophore-labeled oligonucleotide modified nanoparticles or thin films in 0.3 M PBS, to displace the oligonucleotides. After 18 hours at room temperature with intermittent shaking, the solutions containing displaced oligonucleotides were separated from the gold by either centrifugation of the gold nanoparticles, or by removal of the gold thin film. Aliquots of the supernatant were diluted two-fold by addition of 0.3 M PBS, pH 7. Care was taken to keep the pH and ionic strength of the sample and calibration standard solutions the same for all measurements due to the sensitivity of the optical properties of fluorescein to these conditions (Zhao et al., *Spectrochimica Acta* **45A**:1113-1116 (1989)). The fluorescence maxima (measured at 520

nm) were converted to molar concentrations of the fluorescein-alkylthiol modified oligonucleotide by interpolation from a standard linear calibration curve. Standard curves were prepared with known concentrations of fluorophore-labeled oligonucleotides using identical buffer and salt concentrations. Finally, the average number of oligonucleotides per particle was obtained by dividing the measured oligonucleotide molar concentration by the original Au nanoparticle concentration. Normalized surface coverage values were then calculated by dividing by the estimated particle surface area (assuming spherical particles) in the nanoparticle solution. The assumption of roundness is based on a calculated average roundness factor of 0.93. Roundness factor is computed as: (4 x pi x Area)/(perimeter x 2) taken from Baxes, Gregory, *Digital Image Processing*, p. 157 (1994).

J. Quantitation of the Hybridized Target Surface Density.

To determine the activity of attached oligonucleotides for hybridization, fluorophore-labeled oligonucleotides, which were complementary to the surface-bound oligonucleotides (12'F), were reacted with oligonucleotide modified surfaces (gold nanoparticles or thin films) under hybridization conditions (3 μM complementary oligonucleotide, 0.3 M PBS, pH 7, 24 hr). Non-hybridized oligonucleotides were removed from the gold by rinsing twice with buffered saline as described above. Then, the fluorophore-labeled oligonucleotides were dehybridized by addition of NaOH (final concentration ~ 50 mM, pH 11-12, 4 hr). Following separation of the solution containing the 12'F from the nanoparticle solutions by centrifugation, and neutralization of the solutions by addition of 1 M HCl, the concentrations of hybridized oligonucleotide and corresponding hybridized target surface density were determined by fluorescence spectroscopy.

K. Quantitation of Surface Coverage and Hybridization

Citrate stabilized gold nanoparticles were functionalized with 12mer fluorescein-modified alkylthiol DNA (HS-$(CH_2)_6$-5'-CGC-ATT-CAG-GAT-$(CH_2)_4$-F [SEQ ID NO:50]). Surface coverage studies were then performed by thoroughly rinsing away non-chemisorbed oligonucleotides, followed by removal of the fluorophore-labeled oligonucleotides from the